STN/East

#13

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      (FILE 'HOME' ENTERED AT 08:35:58 ON 05 NOV 2002)

      FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:36:04 ON 05 NOV
      2002
L1         34735 S ALCOHOL (1N) DEHYDROGENASE
L2          4751 S L1 AND (MUTA? OR MODIFI?)
L3            32 S L2 AND (ACIDIC)
L4             3 S L3 AND NADH
L5             3 DUP REM L4 (0 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 08:37:43 ON 05 NOV 2002

      FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 08:38:01 ON 05 NOV
      2002
L6             7 S L3 AND INCREAS?
L7             4 DUP REM L6 (3 DUPLICATES REMOVED)
L8             4 S L3 AND ASPARTATE
L9             4 DUP REM L8 (0 DUPLICATES REMOVED)

=> s l1 and brevis
L10           31 L1 AND BREVIS

=> dup rem l10
PROCESSING COMPLETED FOR L10
L11           26 DUP REM L10 (5 DUPLICATES REMOVED)

=> s l11 and (muta? or modif?)
L12            2 L11 AND (MUTA? OR MODIF?)
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(FILE 'HOME' ENTERED AT 09:06:01 ON 05 NOV 2002)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 09:06:05 ON 05 NOV 2002

L1	0	S	BACTOBACILLUS (1N) BREVIS
L2	1646	S	LACTOBACILLUS (1N) BREVIS
L3	29	S	L2 AND (ALCOHOL (1N) DEHYDROGENASE)
L4	24		DUP REM L3 (5 DUPLICATES REMOVED)
L5	2	S	L4 AND DNA

	Type	Hits	Search Text	DBs
1	BRS	5229	alcohol near1 dehydrogenase	USPAT; US-PGPUB; EPO; JPO; DERWENT;
2	BRS	94	brevis and (alcohol near1 dehydrogenase)	USPAT; US-PGPUB; EPO; JPO; DERWENT;
3	BRS	68	(brevis and (alcohol near1 dehydrogenase)) and mutant	USPAT; US-PGPUB; EPO; JPO; DERWENT;
4	BRS	21	((brevis and (alcohol near1 dehydrogenase)) and mutant) and aspartate	USPAT; US-PGPUB; EPO; JPO; DERWENT;
5	BRS	24	((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus	USPAT; US-PGPUB; EPO; JPO; DERWENT;
6	BRS	24	(((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and mutant	USPAT; US-PGPUB; EPO; JPO; DERWENT;
7	BRS	20	((((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and mutant) and aspartic	USPAT; US-PGPUB; EPO; JPO; DERWENT;
8	BRS	24	(((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and brevis	USPAT; US-PGPUB; EPO; JPO; DERWENT;
9	BRS	8	((((brevis and (alcohol near1 dehydrogenase)) and mutant) and lactobacillus) and brevis) and acidic	USPAT; US-PGPUB; EPO; JPO; DERWENT;
10	BRS	0	"79610984"	USPAT; US-PGPUB; EPO; JPO; DERWENT;
11	BRS	10	"796914"	USPAT; US-PGPUB; EPO; JPO; DERWENT;

L5 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1997:667746 CAPLUS
DN 127:289865
TI **Alcohol dehydrogenase** of Lactobacillus and its use in the enzymic production of chiral alcohols
IN Hummel, Werner; Riebel, Bettina
PA Boehringer Mannheim Gmbh, Germany
SO Eur. Pat. Appl., 34 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 796914	A2	19970924	EP 1997-104814	19970320
	EP 796914	A3	19971210		
	R: CH, DE, ES, FR, GB, IT, LI				
	DE 19610984	A1	19970925	DE 1996-19610984	19960321
	JP 10028590	A2	19980203	JP 1997-87644	19970321
	US 6037158	A	20000314	US 1997-822322	19970321
	US 6225099	B1	20010501	US 1999-466109	19991217
PRAI	DE 1996-19610984	A	19960321		
	US 1997-822322	A3	19970321		

OS MARPAT 127:289865
AB An **alc. dehydrogenase** of Lactobacillus that is useful for the prepn. of chiral alcs. from ketones is described. The enzyme purified from **Lactobacillus brevis** had two pH optima (5.5 and 9.0) and a temp. optimum of 50.degree. and a very broad substrate specificity. The gene for the enzyme was cloned by PCR using sequence-derived probes and expressed in Escherichia coli using the com. expression vector pKK177.

L7 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
AN 1993:443992 CAPLUS
DN 119:43992
TI Substitution of Asp-223 residue to Leu in yeast **alcohol dehydrogenase** and coenzyme specificity
AU Lee, Kang Man; Ryu, Ji Won
CS Coll. Pharm., Ewha Womans Univ., Seoul, 120-750, S. Korea
SO Yakhak Hoechi (1992), 36(5), 469-73
CODEN: YAHOA3; ISSN: 0513-4234
DT Journal
LA Korean
AB Yeast **alc. dehydrogenase** (YADH) has an **acidic** residue that interacts with the 2'- and 3'-OH groups of the adenosine ribose moiety of NAD. The **acidic** residue, Asp-223 (according to the horse liver **alc. dehydrogenase** amino acid sequence), is supposed to det. the coenzyme specificity for NAD rather than NADP. Here, Asp-223 was replaced by leucine and the **mutant** YADH was expressed in yeast and characterized for the coenzyme specificity. The turnover nos. of the **mutant** enzyme for NAD and EtOH were decreased 3.5- and 4.8-fold compared to wild-type enzyme, resp. In contrast, the specificity for NADP was **increased** 13-fold. As a result, the **mutant** YADH was able to also employ NADP as a coenzyme.

L7 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2002 ACS
AN 1991:424940 CAPLUS
DN 115:24940
TI An aspartate residue in yeast **alcohol dehydrogenase** I determines the specificity for coenzyme
AU Fan, Fan; Lorenzen, James A.; Plapp, Bryce V.
CS Dep. Biochem., Univ. Iowa, Iowa City, IA, 52242, USA
SO Biochemistry (1991), 30(26), 6397-401
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB In the 3-dimensional structures of enzymes that bind NAD or FAD, there is an **acidic** residue that interacts with the 2'- and 3'-OH groups of the adenosine ribose moiety of the coenzyme. The size and charge of the carboxylate may repel the binding of the 2'-phosphate group of NADP and explain the specificity for NAD. In the NAD-dependent **alc. dehydrogenases**, Asp-223 (horse liver **alc. dehydrogenase** sequence) appears to have this role. The homologous residue in yeast **alc. dehydrogenase** I (residue 201 in the protein sequence) was substituted with glycine, and the D223G enzyme was expressed in yeast, purified, and characterized. The wild-type enzyme was specific for NAD. In contrast, the D223G enzyme bound and reduced NAD and NADP equally well, but, relative to wild-type enzyme, the dissocn. const. for NAD was **increased** 17-fold, and the reactivity (Vmax/Km) on EtOH was decreases to 1%. Even though the catalytic efficiency was reduced, yeast expressing the altered or wild-type enzyme grew at comparable rates, suggesting that equilibration of NAD and NADP pools is not lethal. Thus, Asp-223 participates in binding NAD and in excluding NADP, but it is not the only residue important for detg. specificity for coenzyme.

L12 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 2002:87234 CAPLUS
DN 136:130770
TI Dehydrogenase **mutants** capable of using NAD as coenzyme and their preparation and use for chiral hydroxy compound preparation
IN Riebel, Bettina; Hummel, Werner; Bommarius, Andreas
PA Degussa Aktiengesellschaft, Germany
SO Eur. Pat. Appl., 23 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 1176203	A1	20020130	EP 2001-114953	20010620
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	DE 10037101	A1	20020207	DE 2000-10037101	20000727
PRAI	DE 2000-10037101	A	20000727		

AB The NADH specificity of preferred NADPH-dependent dehydrogenases can be improved by reducing the basicity of the coenzyme binding site through genetic engineering. Dehydrogenases with NADH-dependence suitable for preparative purposes having a kcat/KM value for NAD+ .gtoreq.20 can be obtained with recombinant microorganisms. Thus, the enzyme gene is altered so that basic amino acid(s) in the coenzyme binding site are at least partially replaced by uncharged or neg. charged amino acids. The inventive method is esp. useful for obtaining short-chain dehydrogenases with coenzyme binding sites at the N-terminus. Thus, **mutant** Lactobacillus **brevis alc. dehydrogenases** were prepd. with recombinant Escherichia coli. The **mutant** contg. an aspartic acid at amino acid 38, rather than glycine, showed a 10-fold preference for NAD over NADP.
RE.CNT 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
AN 1999:614147 CAPLUS
DN 131:239737
TI Dehydrogenase **mutants** with improved NAD-dependence, their manufacture with recombinant microorganisms, and their use for chiral hydroxy compound preparation
IN Hummel, Werner; Riebel, Bettina
PA Forschungszentrum Julich G.m.b.H., Germany
SO PCT Int. Appl., 35 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9947684	A2	19990923	WO 1999-DE848	19990318
	WO 9947684	A3	20000323		
	W: JP, US				
	RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	DE 19812004	A1	19990930	DE 1998-19812004	19980319
	EP 1002097	A2	20000524	EP 1999-923384	19990318
	R: AT, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 2001526547	T2	20011218	JP 1999-546407	19990318
	US 6413750	B1	20020702	US 1999-447125	19991118
PRAI	DE 1998-19812004	A	19980319		
	WO 1999-DE848	W	19990318		

AB The NADH specificity of preferred NADPH-dependent dehydrogenases can be

improved by reducing the basicity of the coenzyme binding site through genetic engineering. Dehydrogenases with NADH-dependence suitable for preparative purposes having a kcat/KM value for NAD+ .gtoreq.20 can be obtained with recombinant microorganisms. Thus, the enzyme gene is altered so that basic amino acid(s) in the coenzyme binding site are at least partially replaced by uncharged or neg. charged amino acids. The inventive method is esp. useful for obtaining short-chain dehydrogenases with coenzyme binding sites at the N-terminus. Thus, **mutant** Lactobacillus **brevis alc. dehydrogenases** were prepd. with recombinant Escherichia coli. The **mutant** contg. A9G, G37D, R38L, and K48M substitutions used only NAD as coenzyme..